

# PLANT PATENT APPLICATION

## TITLE

### A NEW BUNASHIMEJI MUSHROOM PLANT NAMED HOKUTO SHIRO ICHIGOUKIN

#### 5 SPECIFICATION

The present plant patent application claims priority of Japanese plant variety application No. 14858, filed July 23, 2002, which is hereby incorporated by reference.

Hokuto Shiro Ichigoukin is a variety of Bunashimeji mushroom plant that was produced by improving the existing varieties to have the following properties: nice white  
10 color, no bitter taste, delicious taste with a higher saccharide content, and cultivatable under similar conditions, including cultivation cycle. The scientific name of the variety is *Hypsizygus marumoreus*. The present plant is an edible mushroom.

#### BACKGROUND OF THE INVENTION

15 The present variety Hokuto Shiro Ichigoukin was produced using mutant fungal strains of two parent varieties, Hokuto Hachigoukin and Hokuto Jyunigoukin, grown by Hokuto Sangyo Kabushiki Kaisha. Specifically, the female variety was Hokuto Hachigoukin and the male variety was Hokuto Jyunigoukin. Hokuto Hachigoukin and Hokuto Jyunigoukin were each submitted to UV irradiation, and then the mutated varieties  
20 were crossbred to produce the present variety Hokuto Shiro Ichigoukin.

The site of development was on property of Hokuto Sangyo Kabushiki Kaisha Kinoko Sougo Kenkyusho in Nagano, Japan.

In April 2000, the mutated Hokuto Hachigoukin was produced, in that a UV-irradiated strain was spawned on a culture medium. In August 2000, the mutated Hokuto  
25 Hachigoukin was harvested, and monospores were isolated.

In parallel, in May 2000, the mutated Hokuto Jyunigoukin was produced, also in that a UV-irradiated strain was spawned on a culture medium. In October 2000, the mutated Hokuto Jyunigoukin was harvested, and monospores were isolated.

In September and October 2000, primary hyphae were obtained from mutated  
30 Hokuto Hachigoukin and mutated Hokuto Jyunigoukin. From December 2000, mutated Hokuto Hachigoukin and mutated Hokuto Jyunigoukin were used to obtain crossbred stains. In February 2001, cultivation was started, and harvesting began in May 2001.

Further, in June 2001, a cultivation test was started for secondary selection. In October 2001, an HM222 strain, which appeared to be a superior variety, was selected. In December 2001, a cultivation test was initiated for stabilization.

5 In January 2002, a characteristic test was started. In May 2002, after repeated cultivation tests, stability, reproducibility and homogeneity, matching the objectives of improvements, were confirmed, i.e., the asexually propagated clones of the present plant are identical to the original plant in all distinguishing characteristics. Thus the HM222 strain was named Hokuto Shiro Ichigoukin.

## 10 SUMMARY OF THE INVENTION

The present invention provides a variety, Hokuto Shiro Ichigoukin, which is an edible mushroom. When this mushroom is cooked in hot water, the bitterness associated with bunashimeji is mostly gone, thus making it a delectable variety. The present variety can be cultivated anywhere a cultivation facility can be arranged.

15 In summary, the morphological characteristics of Hokuto Shiro Ichigoukin are as follows: at the appropriate harvesting period, the cap is small and round; the color of the cap center and margin is white; the thickness and quality of the cap meat are medium; the number of mottled spots is medium; the mottled spots are distributed in the central area; the clarity of the mottled spots is medium; the color of the gills is yellowish white; the  
20 arrangement of the gills is orderly; the width and density of the gills are medium; the length of the stipe is medium; attachment of the stipe to the cap is centric; the shape of the stipe is moderately thick; the color of the stipe is white; there is no hair on the stipe; and the quality of the stipe meat is medium.

25 As to the cultivation data for Hokuto Shiro Ichigoukin, yield is about 118.5 g, the number of effective fruit bodies is 62, and the length of time from spawning to harvesting is 91 days.

## BRIEF DESCRIPTION OF THE DRAWINGS

30 Fig. 1 is a photograph showing a front view of the present plant variety cultivated in vases;

Fig. 2 is a photograph showing a front view of the present variety without growth substrate;

Fig. 3 is a photograph showing a perspective view of the present plant variety cultivated in vases;

Figs. 4a and 4b are photographs showing a surface and a back, respectively, of a culture of the present plant variety;

5 Fig. 5 is a photograph showing a front view of a parent plant variety, Hokuto Hachigoukin, cultivated in vases;

Figs. 6A and 6B are photographs showing a surface and a back, respectively, of a culture of parent plant variety Hokuto Hachigoukin;

10 Fig. 7 is a photograph showing a front view of another parent plant variety, Miyuki M-8171, cultivated in vases; and

Figs. 8A and 8B are photographs showing a surface and a back, respectively, of a culture of parent plant variety Miyuki M-8171.

Fig. 9A and 9B are a table and a graph, respectively, showing comparative mycelial growth of the present plant variety, as compared with that of parent plant  
15 varieties Hokuto Hachigoukin and Miyuki M-8171.

#### DETAILED DESCRIPTION OF THE INVENTION

The characteristics of the present variety will be described in comparison with a corresponding variety, Hokuto Hachigoukin, and with another corresponding variety,  
20 Miyuki M-8171, which is commercially available (samples were bought at a supermarket in Nagano, Japan).

The cultivation site for this comparison was Hokuto Sangyo Kabushiki Kaisha in Nagano, Japan. The cultivation period was January to June 2002. The cultivation was made according to the conventional methods in a facility with a culture room of approximately  
25 60 m<sup>3</sup> in size and a growth room of approximately 30 m<sup>3</sup> in size. In both the culture and growth rooms, an air-conditioning unit, comprising a cooler and heat exchanger, was installed, and in the growth room, cultivation racks and lights were placed. For characteristic analyses, three cultivation tests were performed using 25 bottles per block. The cultivation method used nutrient propagation, and strains were used as seeds.

30 In summary, when compared to Hokuto Hachigoukin, Hokuto Shiro Ichigoukin exhibits the following characteristics: formation of an inhibition zone in dual culture; dense aerial hyphae (Hachigoukin: medium density); cross sectional shape of hat is umbonate (Hachigoukin: umbonate to flat); white cap center (Hachigoukin: yellowish

brown); white cap margin (Hachigoukin: light grayish brown); medium quantity of mottled spots (Hachigoukin: many); central distribution of mottled spots (Hachigoukin: all areas except margin); yellowish white colored gills (Hachigoukin: white); and medium length stipe (Hachigoukin: long).

- 5           When compared to the commercial variety, Hokuto Shiro Ichigoukin exhibits the following characteristics: formation of an inhibition zone in dual culture; dense aerial hyphae (commercial variety: medium density); clumped pattern for fruit body development (commercial variety: grouped pattern); central distribution of mottled spots (commercial variety: all areas except margin); yellowish white colored gills (commercial  
10 variety: white); a diameter under cap to maximum diameter ratio of 1.59 (commercial variety: 1.6-1.8); and a cap diameter to stipe length ratio of  $\geq 2.1$  (commercial variety: 1.5-2.0).

The characteristics of the present plant variety will now be described in details, with relevant comparisons with varieties Hokuto Hachigoukin and Miyuki M-8171.

15   (1) Dual culture

The test was done according to the examination standards established for Shirotamogitake (*Hypsizygus ulmarius*). With regard to the inhibition zone, the results were shown in Table 1 below (+: inhibition zone, -: no inhibition zone formation).

Table 1: Inhibition Zone

Similar variety	Hokuto Shiro Ichigoukin	Hokuto Hachigoukin	Miyuki M-8171
Hokuto Shiro Ichigoukin	-	+	+

20

Thus, an inhibition zone was formed between Hokuto Shiro Ichigoukin and the comparative varieties Hokuto Hachigoukin as well as Miyuki M-8171, which is the closest variety after Hokuto Hachigoukin.

- 25           Photographs of the cultures for the present plant variety and for comparative varieties Hokuto Hachigoukin and Miyuki M-8171 are shown on Figs. 4, 6 and 8, respectively.

(2) Length of culture from spawning to fruit-body-formation promotion

The length of culture from spawning to fruit-body-formation promotion was investigated. Again, the test period was January - June 2002. Except for the number of cultivation days, cultivation was done according to the examination standards established for Shirotamogitake (*Hypsizygus ulmarius*). Three lengths of culture were established at 63, 70 and 77 days. The results are summarized in Table 2 below.

Table 2: Culture Length

Culture length (days)	Growth length (days)	Yield (g/bottle)
63	20.5	128.0
70	20.5	143.4
77	22.0	115.8

Based on the results shown in Table 2, the length of culture was set at 70 days because the yield was the highest for this length.

(3) Optimal budding temperature

To determine the optimal budding temperature, cultivation was done for 70 days according to the examination standards established for Shirotamogitake (*Hypsizygus ulmarius*). Three budding temperatures were established as shown in Table 3 below.

Table 3: Optimal Budding Temperature

Budding temperature (°C)	Budding length (days)
13 ~ 14	6.7
14 ~ 15	6.3
15 ~ 16	6.9

Based on the results shown in Table 3, the minimal temperature was set at 14-15°C because the length of budding was the shortest at this temperature.

(4) Optimal growth temperature

The optimal growth temperature was studied through cultivation for 70 days according to the examination standards established for Shirotamogitake (*Hypsizygus ulmarius*). Three growth temperatures were established as shown in Table 4 below.

Table 4: Growth Temperature

Growth temperature (°C)	Growth length (days)	Yield
13 ~ 14	22.1	115.6
14 ~ 15	21.2	121.1
15 ~ 16	24.9	107.1

Based on the results shown in Table 4, the optimal growth temperature was set at 14-15°C because the yield was the greatest at this temperature.

(5) Summary of cultivation characteristics

The cultivation characteristics of the present plant variety are shown in Table 5 below.

Table 5: Summary of Cultivation Characteristics

Characteristics	Hokuto Shiro Ichigoukin	Hokuto Hachigoukin	Miyuki M- 8171
Length of culture from spawning to fruit-body-formation promotion (days)	70	70	70
Length of time from fruit-body-formation promotion to fruit body harvesting at optimal temperature (days)	21	21	21
Optimal budding temperature (°C)	14 ~ 15	14 ~ 15	14 ~ 15
Fruit body development pattern	Clumped	Clumped	Group
Yield (g/bottle)	118.5	117.1	105.1
Number of effective fruit bodies	62	100	79
Medium compatibility	Sawdust	Sawdust	Sawdust
Tree selectivity	Mixture	Mixture	Mixture
Cap Size (mm)	23.2	23.4	24.9
Cross sectional shape	Umbonate	Umbonate	Umbonate
Center color	White	Yellowish brown	White
Margin color	White	Light grayish brown	White
Meat thickness (mm)	8.1	6.8	7.4
Meat quality	Medium	Medium	Medium
Mottled spot quantity	Medium	Many	Medium
Mottled spot size	Medium	Medium	Medium
Mottled spot distribution	Central area	All areas except margin	All areas except margin
Mottled spot clarity	Medium	Medium	Medium
Cracking	Absent	Absent	Absent
Gills Color	Yellowish white	White	White
Arrangement	Orderly	Orderly	Wavy and frizzled
Width	Medium	Medium	Medium
Density	Medium	Medium	Medium
Stipe Length (mm)			
Attachment to cap	Centric: eccentric	Centric: eccentric	Centric: eccentric

Shape	Medium thick	Medium thick	Medium thick
Color	White	White	White
Hair	Absent	Absent	Absent
Diameter under cap to maximum diameter ratio			
Cap diameter to stipe length ratio			
Meat quality	Medium	Medium	Medium

(6) Cultivation characteristic data

Detailed cultivation characteristics of the present plant variety are shown in Table 6 below.

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Table 6: Detailed Cultivation Characteristics

Characteristics	Characteristic code of filed variety										Note (Data)	Code of the similar variety	
	01	02	03	04	05	06	07	08	09	Hokuto Hachigoukin		Miyuki M-8171	
Cultivation characteristics													
Length of culture from spawning to fruit-body formation promotion (days)	40 and under	41~50	51~60	61~70	71~80	81~90	91~100	101~110	111 and over	70days	04	04	
Length of time from fruit-body-formation promotion to fruit body harvesting at optimal temperature (days)			19 and under	20~25			26 and over			21days	05	05	
Optimal budding temperature ( C )	10 and under	11~14	15~18	19~22	23~26	27 and over				14-15	03, 04	03, 04	
Optimal developed temperature ( C )	8 and under	9~11	12~14	15~17	18~20	21 and over				14-15	04, 05	04, 05	
Optimal intensity													
Budding period			Early		Intermediate		Late						
Fruiting habit	Grouped	Scattered	Clumped						other	Clumped	03	01	
Yield (g/bottle)		80 and under	81~100	101~120			121 and over			118.5	06	06	
Number of effective fruit bodies		20 and under	21~25	26~30	31~35	36~40	41 and over			62.0	07	07	
Medium compatibility	Log wood	Sawdust							other	Sawdust	02	02	
Tree selectivity	Broad leaved	Coniferous	Mixture						other	Mixture	03	03	

Characteristics	Characteristic code of filed variety									Note (Data)	Code of the similar variety	
	01	02	03	04	05	06	07	08	09		Hokuto Hachigoukin	Miyuki M-817
Morphology												
Cap												
Size			small		medium		large			23.2(mm)	03	03
Cross sectional shape	Convex	Umbonate	expanded	infundibuliform					other	Umbonate	02, 03	02
Center color	W	GY	LG B	YB	GB	DY B	DGY B	DG B	other	White	04 9YR1916	01 No4-01
Margin color	W	G-Y	LG B	YB	GB	DY B	DGY B	DG B	other	White	03 9YR1915	01 No4-01
Thickness			Thin		medium		Thick			8.1(mm)	05	05
Hardness			Soft		Medium		Hard			Medium	05	05
Mottled spot quantity			Few		Medium		Many			中	07	05
Mottled spot size			small		medium		large			中	05	05
Mottled spot distribution	Central area	All areas except margin							other	Central area	02	02
Mottled spot clarity			unclear		medium		Clear			Medium	05	05
Cracking	Absent								Present	Absent	01	01
Gills												
Color	W	YW	LOY	GY					other	Yellowish white No. 4-2501	01	01
Arrangement	Orderly	Sinusate							other	Orderly	01	02



Characteristics	Characteristic code of filed variety									Note (Data)	Code of the similar variety	
	01	02	03	04	05	06	07	08	09		Hokuto Hachigoukin	Miyuki M-817
Width			Narrow		Medium		Wide			Medium	05	05
Density			Spaced		Medium		Dense			Medium	05	05
Stipe												
Length			Short		Medium		Long			49.3(mm)	07	03
Attachment to cap	Centric	Eccentric							Other	Centric 7: eccentric 3	01	01
Shape	Thin Long	Thin Short	Thick Long	Thick Short	Medium Thick				Other	Medium thick	05	05
Color	White								Colored	White	01	01
Hair	Absent								Present	Absent	01	01
Diameter under cap to maximum diameter			1.5 and under		1.8~ 1.8		1.8 and over			1.59	03	05
Cap diameter to stipe length ratio			1.4 and under		1.5~ 2.0		2.1 and over			2.1	07	05
Hardness			Soft		Medium		Hard			Medium	05	05
Others characteristics												

Characteristics	Characteristic code of filed variety									Note (Data)	Code of the similar variety	
	01	02	03	04	05	06	07	08	09		Hokuto Hachigoukin	Miyuki M-8171
Genetic												
Dual culture												
Inhibition zone	—								+	01	09	09
Physiology												
Mycelial growth												
Effect of temperature												
The optimum(°C)	22	23	24	25	26	27	28	29	30	24°C	04	03
Growth rate												
5°C										4.0mm/day	4.9	6.4
10°C										9.3mm/day	12.2	13.7
15°C										19.9mm/day	18.7	20.5
20°C										25.8mm/day	24.3	26.3
25°C										28.6mm/day	30.6	31.2
30°C										11.6mm/day	15.5	16.9
35°C										0.8mm/day	2.6	1.2
Mycelial density			Thin		medium		Thick			Thick	05	05
Development of aerial hyphae			Few		medium		Many			Medium	03	05